World Journal of Agricultural Sciences 6 (5): 555-564, 2010
ISSN 1817-3047
© IDOSI Publications, 2010

Bacteria from Sand Dunes of Goa Promoting Growth in Eggplant

[1]Aureen Godinho, [2]R. Ramesh and [1]Saroj Bhosle

[1]Department of Microbiology, Taleigao Plateau, Goa University, Goa - 403 206, India
[2]Indian Council of Agricultural Research (ICAR), Ela, Old Goa - 403 402, India

Abstract: The present study is important in view of identification of a native bacterial strain with a strong potential for development as a bioinoculant. The use of PGPR as natural fertilizers is advantageous for the development of sustainable agriculture given the negative impact of chemical fertilizers. An organism with properties such as phosphate solubilization, disease control potential and rhizosphere colonization would seem ideal for selection as well as a suitable bioinoculant. In this study among the four sand dune bacterial isolates, *B. subtilis*, *K. rosea* and *M.arborescens* were found to be good plant growth promoters in neutral soil conditions. All the four sand dune bacterial cultures were found to have ACC deaminase activity and other attributes like IAA, HCN production, siderophore production and phosphate solubilization. Also ACC deaminase activity might have produced better root growth in the initial stages of crop growth by reducing the level of ethylene in the roots of the developing plants thereby increasing the root length and growth. This resulted in healthy plant due to balanced nutrient availability and uptake, which in turn increased plant biomass. Although ACC deaminase activity in enhancing plant growth cannot be ruled out, coordinated expression of multiple growth promoting traits could have been responsible in the overall plant growth promotion of eggplant by these sand dune bacterial isolates. The present study has therefore confirmed the bioprospects of using these sand dune bacteria as biofertilizers for agricultural crops.

Abbreviations: IAA - indole-3- acetic acid; DAS - days after sowing; SDB - sand dune bacteria, PGPR- plant growth-promoting rhizobacteria - (HCN) Hydrogen cyanide, ACC (1-aminocyclopropane carboxylate)

Key words: Eggplant · Growth promotion · Sand dune bacteria · Rhizobacteria · Rhizosphere · Endophytic

INTRODUCTION

Sand dunes are mounds of drifted sand on the beach topped with vegetation. Sand dune systems have limiting levels of major nutrients notably of nitrogen, phosphorus and potassium. The shifting sands, saline conditions, lack of humus, high temperatures and deep ground water makes it difficult for vegetation to grow. Only special type of plants adapt to these adverse conditions [1]. The accumulation of humus results in improved moisture and nutrient holding capacity of developing dune soils. The ability of the dune soils to retain water and mineral nutrients rise as organic matter content increases [2].

In the rhizosphere, bacteria are abundantly present, most often organized in microcolonies. Some of these rhizobacteria not only benefit from the nutrients secreted by the plant root but also beneficially influence the plant in a direct or indirect way, resulting in a stimulation of its growth. These plant growth-promoting rhizobacteria (PGPRs) can be classified according to their beneficial effects. For instance, biofertilizers can fix nitrogen, which can subsequently be used by the plant, thereby improving plant growth when the amount of nitrogen in the soil is limiting. Phytostimulators can directly promote the growth of plants, usually by the production of hormones. Biocontrol agents are able to protect plants from infection by phyto-pathogenic organisms. There is considerable experimental support for the idea that plant growth promoting bacteria may be used as biofertilizers or biological disease control agents to increase agricultural yields [3]. The large-scale application of PGPRs to crops as inoculants would be attractive as it would substantially reduce the use of chemical fertilizers and pesticides, which often pollute the environment. In addition, the application of PGPRs would increase crop yield, thereby helping to feed the growing world population [4].

Corresponding Author: Dr. Saroj Bhosle, Professor, Department of Microbiology,
Goa University, Taleigao Plateau, Goa - 403206, India.
Tel: +0832-6519080 Extn: 231, E-mail: sarojbhosle@yahoo.co.in.

Our studies have shown that there are a diverse group of bacteria flourishing in this sand dune ecosystem which have the potential to be used as a bio-resource. This study is an attempt to assess their role as a plant growth promoter in a vegetable crop, eggplant.

MATERIALS AND METHODS

Isolation of Rhizosphere and Endophytic Bacteria from Sand Dune Vegetation: Rhizosphere sand from *Ipomoea pes caprae* and *Spinifex littoreus*, sand dune vegetation was collected and the sand was dispensed in 0.85% saline and dilutions were prepared. The dilutions were further plated onto nutrient agar medium (peptone, 5 g/l; beef extract, 3 g/l; sodium chloride, 1.5 g/l; agar, 15 g/l) for the total viable count, while polypeptone yeast extract glucose agar medium (peptone, 5 g/l; yeast extract 1.5 g/l; disodium hydrogen phosphate, 1.5 g/l; sodium chloride, 1.5 g/l; magnesium chloride, 0.1 g/l; glucose, 10%; sodium carbonate, 10%; agar, 15 g/l) was used for isolating alkaliphiles. The nutrient agar and polypeptone yeast extract glucose agar plates were incubated at 28 ± 2°C for 48 hrs for the development of colonies. The endophytic bacteria of the vegetation were isolated by taking one gram of roots and washing it well in sterile distilled water. The roots were then treated with 0.01 M EDTA and centrifuged at 5,000 rpm for 10 mins and this process was repeated 3 times to remove any sand particles attached to the root surface. The roots were then transferred to a sterile motar and homogenized. The extract obtained was diluted upto 10^{-6} and the dilutions were plated on respective media as mentioned above for isolation.

Characterization of the Isolated Bacteria: Based on the ability of the bacterial isolates to produce industrially important enzymes (protease, cellulase, amylase, lipase, tannase), siderophores (iron chelators) and inorganic phosphate solubilizers, the most effective cultures were identified based on standard biochemical methods using Bergeys Manual of Systematic Bacteriology [5, 6] and sequencing of 16s rRNA. PCR amplification of almost full length 16S rRNA gene was carried out with eubacteria specific primerset16F27N(5'CCAGAGTTTGATCMTGGCTCAG3') and 16R1525XP(5'TTCTGCAGTCTAGAAGGAGGTG WTCCAGGC-3'). The PCR was performed in an automated Gene Amp PCR system 9700 thermal cycler (Applied Biosystems, Foster City, USA) The sample was sequenced by using BIG DYE Terminator cycle sequencing ready reaction kit (v3.1) in ABI PRISM 3730 Genetic Analyzer (Applied Biosystems, USA).

Screening for Enzymes, Siderophores, Phosphate Solubilization Activity of the Isolates: The bacterial isolates were screened for the production of Protease (skimmed milk agar medium), Cellulase (carboxymethylcellulose agar medium), Amylase (Starch agar medium), Tannase (tannin degrading agar medium) and Lipase (Tributyrin agar medium) [7]. The bacterial isolates were further screened for siderophore production by plate assay [8]. The phosphate solubilization activity of the isolates was checked by streaking cultures on P solubilizing media (Pikosvkya agar) containing tricalcium phosphate as carbon source [9]. Based on *in vitro* results the promising isolates viz. *Microbacterium arborescens, Kocuria rosea, Bacillus* sp MF-A4 and *Bacillus subtilis* were used for further experiments.

Production of indole acetic acid (IAA), Hydrogen cyanide (HCN) and screening for ACC (1-aminocyclopropane carboxylate) deaminase activity of the isolates: Production of indole acetic acid: The isolates were grown in nutrient both containing 0.5% tryptophan for 30 hrs. One ml of cell free culture filtrate was reacted with two ml of Salkowsky's reagent (50ml of 35%perchloric acid, 1 ml 0.5M FeCl3) and incubated for 30 mins and the absorbance was read at 530nm [10]. IAA was used as standard and presence of IAA in the culture filtrate was quantified as μg/ml.

Production of Hydrogen cyanide (HCN): The isolates were spread plated on respective media supplemented with 4.4 g /l- of glycine. Filter paper strips soaked in picric acid solution were placed in the lid of each plate. Plates were sealed with parafilm and incubated for 72h. Production of HCN was indicated by the change in colour of the filter paper strips from orange to red colour. The intensity of the colour was recorded visually [3, 7].

Screening for ACC Deaminase Activity: Bacterial isolates were screened for the ability to use ACC (1-aminocyclopropane carboxylate, Sigma chemical) as a sole nitrogen source. The isolates were streaked on Dworkin and Foster (DF salts) minimal medium containing 3.0 mM solution of filter sterilized ACC as the sole source of nitrogen. The inoculated plates were incubated at 28oC and plates were observed for growth [3, 7].

Testing the Growth Promoting Effect of the Sand Dune Bacteria (SDB) on Eggplant by the Roll Towel Method: 24h old grown cultures were centrifuged at 8,000 rpm for 10min and the cell pellet was suspended in phosphate buffered saline (pH 7.0) and mixed with sterile talc powder to form slurry. Eggplant seeds of Agassaim cultivar were coated with the slurry and placed on the germination paper. The paper was loosely rolled and tied with thread and placed in a beaker of water (2cm depth). The cell pellets of the four culture isolates were mixed together in equal proportions to form the consortium and the seeds were treated as above. After a week, the parameters like the number of seeds germinated, shoot length and root length were recorded. In order to test the effect of the culture on pre-germinated eggplant seeds, the seeds were allowed to germinate for 3 days and then treated in a similar way as mentioned above.

Testing the Growth Promoting Efficacy of Sand Dune Rhizobacteria under *in Vivo* Conditions: An experiment was conducted in split plot design where soil sterilization was main treatment and bacterial isolates were sub-treatments. *B. subtilis* was grown in nutrient broth at pH 7 while *M. arborescens*, *Bacillus sp. MF-A4* and *K. rosea* were grown in poly peptone yeast extract glucose broth at pH 10. Seeds of eggplant cultivar Agassaim were soaked in the 48 hr old grown cultures of the above mentioned isolates for 30 mins and were allowed to dry under shade for 30 min. The treated seeds (150) were sown in pot mixture that was treated with 48 hr old grown culture of the respective isolates to a final concentration of 5 percent. The four culture isolates were mixed together in equal proportions to form the consortium. Suitable control was maintained without any treatment while a positive control was also maintained with the recommended dose of fertilizers and regular crop production practices were followed. Observations were recorded at frequent intervals on number of germinated seedlings; shoot length, root length and plant wet weight up to the seedlings attained growth stage suitable for transplanting. In an another experiment, the normal non-sterile field soil was only used in pots and all the isolates were grown at pH 7.0 and the experiment was conducted as described above. This experiment was repeated under same conditions once again. All the data were statistically analyzed using ANOVA.

Change in Population of Sand Dune Bacteria Inoculated: Soil samples were collected from the pots on day one and 35 days after sowing to check the population of bacteria present in the soil. The dilutions were plated on nutrient agar, pH 7.0 to observe the population of *B. subtilis* while polypeptone yeast extract glucose agar, pH 10.0 was used to identify the *M. arborescens*, *K. rosea* and *Bacillus sp.* MF-A4. The isolates were identified based on their pigment production and colony morphology.

Change in the Fertility Levels of Soil Inoculated with Sand Dune Bacteria: Soil samples were taken from the pots on day one immediately after the treatments and 35 days after sowing to analyze carbon content, available nitrogen and phosphate content of the soils. Carbon content of the soils was determined using the Walkley and Black's rapid titration method [11]. Available nitrogen content of soil was estimated using alkaline potassium permanganate method and phosphate content of soil was determined by the Olsen method [12].

RESULTS

Isolation of Sand Dune Bacteria and Characterization of Promising Isolates: Four hundred bacterial isolates were isolated from the sand dune vegetation of Goa, India. Rhizosphere soil and roots of the vegetation (Ipomoea pes caprae & Spinifex littoreus) were collected from Miramar, Aswem and Mandrem beaches of North Goa. The effective bacterial isolates were identified using 16S r RNA sequencing. The identified bacterial isolates viz. Bacillus subtilis, (Gene bank Accession no DQ287964), Microbacterium arborescens (Gene bank Accession no DQ287961), Bacillus sp. MF-A4 (Gene bank Accession no DQ287962) and Kocuria rosea (Gene bank Accession no DQ287963) were used for further experiments to assess the growth promoting ability in eggplant. M. arborescens is a gram positive rod, facultative alkaliphile; K. rosea is gram positive cocci, facultative alkaliphile; Bacillus sp. MF A4 is a gram positive sporulating rod, facultative alkaliphile and B.subtilis is a gram positive rod, neutrophile. All the selected isolates produced protease, amylase, cellulase, tannase and lipase enzymes. Siderophore production and phosphate solubilization were observed in all the isolates. IAA production was detected and it was found to range between 26-50 μg/ml. Bacillus subtilis showed the highest IAA production (50 μg/ml) followed by M. arborescens (33 μg/ml), Bacillus sp. MF-A4 (30 μg/ml) and Kocuria rosea (26 μg/ml). M. arborescens and Bacillus sp. MF-A4 showed intense orange colour indicating maximum HCN production and B. subtilis showed light orange colour while Kocuria rosea was not found to produce HCN. All the cultures had the ability to grow in minimal medium with ACC as the sole nitrogen source.

Table 1: Effect of sand dune bacteria on germination of eggplant seeds

S.No	Sand dune bacteria (SDB)	Seed treatment with SDB		Pre-germinated seeds treated with SDB	
		Shoot length (cm)[a]	Root length (cm)[a]	Shoot length (cm)[a]	Root length (cm)[a]
1	*Bacillus subtilis*	6.97 ± 0.20	4.22 ± 0.12	6.69 ± 0.23	6.17 ± 0.18
2	*Microbacterium arborescens*	5.97 ± 0.80	4.11 ± 0.30	6.50 ± 0.22	6.77 ± 0.23
3	*Kocuria rosea*	8.01 ± 0.31	4.62 ± 0.03	6.89 ± 0.11	8.22 ± 0.07
4	*Bacillus sp. MF-A4*	8.13 ± 1.05	4.28 ± 0.14	6.68 ± 0.15	6.83 ± 0.411
5	Consortium	6.46 ± 0.21	4.42 ± 0.13	6.59 ± 0.10	6.00 ± 0.46
6	Control	6.38 ± 0.31	4.53 ± 0.18	6.51 ± 0.20	5.91 ± 0.28
	CD(0.05)	1.51	[b]NS	0.211	1.012
	CV	14.4	7.86	2.104	10.09

[a]Mean of triplicates, [b]NS- not significant

Table 2: Growth promoting effects of sand dune bacteria in sterilized and non-sterilized soil on eggplant seedlings

S.No	Treatments	No. of seeds germinated / 150 seeds (8DAS[e])		Growth at 25 DAS: Shoot length[c,f]		Root length[c,f]		Wet weight[d,f]	
		[a]I	[b]II	I	II	I	II	I	II
1	*B.subtilis*	86.0	80.0	5.34	3.49	4.03	2.77	0.93	0.73
2	*M.arborescens*	52.5	48.5	4.81	4.13	3.09	2.58	1.05	0.68
3	*K.rosea*	75.0	80.0	4.85	4.67	2.44	3.04	1.33	1.07
4	*Bacillus sp. MF A-4*	59.0	41.0	5.30	4.25	2.22	2.63	1.84	0.86
5	Consortium	67.5	70.0	4.91	4.70	3.10	2.11	1.92	1.24
6	Control	70.0	51.5	5.36	4.29	3.77	3.07	1.82	1.09
7	+ve control	26.5	64.0	6.17	3.23	4.73	2.72	1.82	1.16
	CD (0.05)								
	Main	11.8		5.09		1.89		1.94	
	Sub	17.9		1.08		0.68		0.42	
	MXS	25.4		1.52		0.96		0.59	

S.No	Treatments	Growth at 30 DAS: Shoot length		Root length		Wet weight		Growth at 35 DAS: Shoot length		Root length		Wet weight	
		I	II	I	II	I	II	I	II	I	II	I	II
1	*B.subtilis*	8.07	6.82	4.96	2.40	2.10	1.42	9.50	7.90	4.40	2.85	3.08	2.80
2	*M.arborescens*	8.55	7.50	3.90	2.55	2.24	2.25	9.10	9.20	5.80	2.75	3.38	1.78
3	*K.rosea*	9.30	10.35	4.00	4.35	2.16	1.79	8.15	8.45	7.35	4.40	5.47	2.94
4	*Bacillus sp. MF A-4*	9.20	7.13	4.00	2.82	2.57	2.55	10.60	9.60	4.20	4.35	2.33	2.27
5	Consortium	7.60	9.00	4.05	2.85	2.25	2.22	9.10	9.85	4.50	3.65	1.90	2.65
6	Control	6.80	6.70	4.25	3.45	1.45	2.51	7.00	7.45	6.50	4.30	2.09	2.15
7	+ve control	7.20	6.76	4.50	3.50	1.46	2.61	7.10	11.70	6.00	2.90	2.20	5.79
	CD (0.05)	2.60		0.86		3.18		2.36		3.36		0.51	
	Main	1.96		0.84		0.98		1.54		1.06		0.83	
	MXS	2.77		1.20		1.39		1.17		1.41		1.17	

[a]I: Non-sterilized soil

[b]II: Sterilized soil

[c]Shoot length, Root lengths are in cm

[d]Wet weight of 10 plants at 25 DAS and 5 plants at 30 DAS, 35DAS (g)

[e]DAS - Days after sowing

[f]Mean of triplicates

Growth Promoting Effect of the SDB on Eggplant by the Roll Towel Method: When the seeds were treated with the talc slurry the highest shoot length was recorded in *Bacillus sp. MF-A4* (8.13 cm) and *K. rosea* (8.01 cm) treatments and the root length was highest (4.62 cm) in *K. rosea*. When germinated seeds were treated with talc slurry the highest shoot length (6.89 cm) and root length (8.22 cm) was recorded in *K. rosea* (Table 1).

Growth Promoting Ability of Sand Dune Bacteria in Eggplant Nursery

In Sterile and Non-Sterile Soil: Among sterilized and non sterilized soil, there was no significant difference except in three parameters (root length at 30 DAS, shoot length and wet weight at 35 DAS). The highest number of seeds germinated in *B. subtilis* treatment (86.0 and 80.0 in non-sterilized and sterilized soil) (Table 2).

Growth at 25 DAS: In non-sterilized soil, positive control recorded maximum shoot and root length (6.17 cm and 4.73 cm). In sterilized soil, consortium treatment recorded maximum shoot length (4.7 cm) and control recorded maximum root length (4.29 cm). Consortium treatment recorded maximum plant weight (1.92g and 1.24g) both in non-sterilized and sterilized soil in both the cases (Table 2).

Growth at 30 DAS: In non-sterilized soil, *K. rosea and Bacillus sp.* MF-A4 recorded the maximum shoot length (9.3 cm & 9.2 cm). *B. subtilis* recorded the maximum root length (4.96 cm). In sterilized soil, *K. rosea* recorded the maximum shoot and root length (10.35 & 4.35 cm). *Bacillus sp.* MF-A4 recorded the maximum plant weight both in sterilized (2.57 g) and non-sterilized (2.55 g) soil conditions(Table 2).

Growth at 35 DAS: In non-sterilized soil, *Bacillus sp.MF-A4* recorded the maximum shoot length (10.6 cm) and *K. rosea* recorded maximum root length (7.35 cm) and plant weight (5.47 g). In sterilized soil, consortium treatment recorded maximum shoot length (9.6 cm) and *K. rosea* recorded maximum root length (4.4 cm) and plant weight (2.94 g) (Table 2).

From this experiment, it was observed that there was no significant difference between sterilized and non-sterilized soil on the eggplant growth, when the bioinoculants were used. Hence the second experiment was carried out using only normal field soil.

Growth Promoting Ability of Sand Dune Bacteria Grown at Neutral pH under Normal Soil Conditions

Growth at 30 DAS: Growth at 30 DAS: *B. subtilis* and *K. rosea* recorded maximum shoot length in the first (11.68 cm & 11.08 cm) and second (10.52 cm and 10.43 cm) experiment. Consortium treatment recorded maximum root length (4.74 cm) in the first experiment while *B. subtilis*(5.47 cm) in the second experiment. *K. rosea* recorded maximum plant weight (3.86 g) in the first experiment while *B. subtilis* (5.68 g) in the second experiment (Table 3).

Growth at 37 DAS: *Bacillus sp.* MF-A4 and consortium treatment recorded the maximum shoot length in the first (14.40 cm and 13.42 cm) experiment while *B. subtilis* and *K. rosea*(12.8 cm and 12.4 cm) in the second experiment. Consortium treatment and *Bacillus sp. MF-A4* recorded maximum root length (7.70 cm and 6.52 cm) in the first experiment whereas *B. subtilis* and consortium (8.4 cm and 8.34 cm) in the second experiment. *Bacillus sp.MF-A4* and consortium treatments recorded maximum plant weight (5.47 g) in the first experiment while *B. subtilis* and *K. rosea*(8.30 g and 6.88 g) in the second experiment(Table 3).

Growth at 44 DAS: *K. rosea* and *M. arborescens* recorded the maximum shoot length (13.8 cm and 13.59 cm) in the first experiment while *B. subtilis* and *K. rosea* (14.36 cm &14.08 cm) in the second experiment. *Bacillus sp.* MF-A4 and *M. arborescens* recorded maximum root length (8.56 cm and 7.54 cm) in the first experiment while positive control and control (9.55 cm and 8.33 cm) in the second experiment. Control and *M. arborescens* recorded maximum plant weight (5.58 g and 4.03 g) in the first experiment while *Bacillus sp MF-A4* and *M. arborescens* (9.72 g and 8.73g) in the second experiment (Table 3).

Change in Population of Sand Dune Bacteria Inoculated to Soil: In general as compared to the population of bacteria present in soil on inoculation there was a reduction in population at the end of the experiment in sterile and non sterile soil conditions. Among the four bacterial inoculants *B. subtilis* was found to show greater survival ability in both sterile and nonsterile soil; and the population was found to be maintained at 10^6cfu/g after 35 days of inoculation into the soil(Table 4). Comparatively, *M. arborescens* was at lower population of 10^4 cfu/g in non sterile soil and 10^3 cfu/g in sterile soils. *K. rosea* was at 10^4 cfu/g in non sterile and 10^5 cfu/g in sterile soil while *Bacillus sp MF-A4* survived at 10^5 cfu/g

Table 3: Growth promoting effects of sand dune bacteria in normal soil on eggplant seedling

		Growth at 30 DAS								
		Shoot length*			Root length*			Wet weight*		
S.No	Treatments	I	II	Mean	I	II	Mean	I	II	Mean
1	*B.subtilis*	11.68	10.52	11.10	3.52	5.47	4.49	2.48	5.68	4.08
2	*M.arborescens*	10.19	10.17	10.18	4.53	4.54	4.53	2.41	4.71	3.56
3	*K.rosea*	11.08	10.43	10.75	3.20	4.78	3.99	3.86	4.97	4.41
4	*Bacillus sp.MF A-4*	10.4	9.72	10.06	4.47	4.55	4.51	2.55	4.89	3.72
5	Consortium	10.61	9.88	10.24	4.74	5.03	4.88	2.24	4.54	3.39
6	Control	7.12	8.65	7.88	2.75	4.82	3.78	2.1	2.94	2.52
7	+ve control	7.94	9.36	8.65	4.01	4.74	4.37	1.64	3.96	2.80
	CD (0.05)	2.21	1.044		NS	NS		NS	NS	
	CD (0.01)	3.10								
		Growth at 37 DAS								
		Shoot length*			Root length*			Wet weight*		
S.No	Treatments	I	II	Mean	I	II	Mean	I	II	Mean
1	*B.subtilis*	11.79	12.8	12.29	4.23	8.406	6.31	1.69	8.30	4.99
2	*M.arborescens*	11.35	13.0	12.17	4.30	7.66	5.98	2.06	4.06	3.06
3	*K.rosea*	12.81	12.4	12.6	5.43	8.20	6.81	3.83	6.88	5.35
4	*Bacillus sp.MF A-4*	14.40	11.9	13.15	6.52	6.533	6.52	5.47	5.90	5.68
5	Consortium	13.42	11.6	12.51	7.70	8.34	8.02	5.47	6.06	5.76
6	Control	11.60	10.5	11.05	3.56	7.22	5.39	4.03	3.55	3.79
7	+ve control	10.21	11.7	10.95	6.34	8.12	7.23	2.56	5.52	4.03
	CD (0.05)	1.72	1. 32		1.34	NS		2.38	NS	
	CD (0.01)	2.41			1.88					
		Growth at 44 DAS								
		Shoot length*			Root length*			Wet weight*		
S.No	Treatments	I	II	Mean	I	II	Mean	I	II	Mean
1	*B.subtilis*	12.15	14.36	13.25	6.27	7.68	6.97	2..20	9.00	5.60
2	*M.arborescens*	13.59	13.94	13.76	7.54	7.20	7.37	4.03	8.73	6.38
3	*K.rosea*	13.8	14.08	13.94	6.48	6.90	6.69	3.18	8.29	5.73
4	*Bacillus sp.MF A-4*	13.17	13.48	13.32	8.56	7.20	7.88	3.31	9.72	6.51
5	Consortium	13.04	13.41	13.22	7.17	7.53	7.35	3.67	6.43	5.05
6	Control	11.99	12.03	12.01	4.89	8.33	6.61	5.58	6.52	6.05
7	+ve control	10.57	13.68	12.12	7.13	9.55	8.34	2.64	8.35	5.49
		1.65	1.332		NS	NS		NS	NS	

*Mean of triplicates

Table 4: Change in total viable count of sand dune bacteria inoculated under nursery pot conditions in sterilized and nonsterilized soil conditions (NS- non sterilized soil, SS- sterilized soil)

	Total viable counts (cfu/g of soil)		
Sand dune bacteria	Day 1	35 DAS (NS)	35 DAS (SS)
Bacillus subtilis	$1.4x\ 10^{9}$	3.4×10^{6}	5.88×10^{6}
M. arborescens	4.400×10^{11}	2×10^{4}	4.0×10^{3}
K. rosea	2.60×10^{10}	6×10^{4}	8.20×10^{5}
Bacillus sp MF-A4.	5.40×10^{10}	2.6×10^{5}	2.00×10^{4}
Consortium	1.25×10^{8}	1.20×10^{4}	-

Table 5: Change in the soil properties of sterilized and non sterilized soils inoculated with sand dune bacteria

		pH		Carbon content %		Available N content %		PO_4- P content (mg/kg soil)	
Treatments		Day 1	Day 35	Day 1	Day 35	Day 1	Day 35	Day 1	35 DAS
B. subtilis	[a]NS	6.75	7.30	0.012	0.042	0.020	0.033	30	50
	[b]SS	6.60	7.20	0.011	0.039	0.020	0.031	28	48
M. arborescens	NS	6.53	7.25	0.012	0.032	0.023	0.045	25	35
	SS	6.44	7.20	0.011	0.022	0.021	0.042	24	34
K. rosea	NS	6.03	7.50	0.012	0.045	0.025	0.054	24	32
	SS	6.01	7.56	0.012	0.040	0.024	0.053	22	30
Bacillus sp.MF-A4	NS	6.43	7.23	0.013	0.064	0.035	0.052	28	40
	SS	6.33	7.32	0.014	0.059	0.032	0.051	26	38
Consortium	NS	6.54	7.21	0.014	0.020	0.025	0.048	32	46
	SS	6.60	7.25	0.012	0.021	0.024	0.046	30	44
Control	NS	6.80	6.85	0.012	0.013	0.020	0.030	30	31
	SS	6.78	6.9	0.014	0.012	0.023	0.028	28	30

[a]NS - Non-sterile soil

[b]SS- Sterile soil

Table 6: Change in soil properties due to treatments with SDB grown at neutral pH

	pH		Carbon content %		Available nitrogen %		PO4-P(mg/ kg soil)	
Treatments	Day 1	46 DAS	Day 1	46 DAS	Day 1	46 DAS	Day 1	46 DAS
B. subtilis	6.02	6.49	0.014	0.013	0.0035	0.0035	34	49
M. arborescens	6.32	6.54	0.014	0.023	0.0045	0.0035	30	36.5
K. rosea	6.13	7.62	0.015	0.018	0.0035	0.0055	32	37
Bacillus sp MF-A4.	6.16	7.06	0.016	0.03	0.003	0.005	29	47
Consortium	6.55	7.44	0.009	0.015	0.006	0.002	31	50.5

in nonsterile and 10^4 in sterile soil conditions. Consortium survived at 10^4 cfu/g in nonsterile soil conditions and was not detected in sterile soil conditions after 35 DAS.

In normal soil conditions, where pH of the growth medium was maintained at pH 7, *Bacillus sp MF A4, B. subtilis, K. rosea* and *M. arborescens* showed a consistent survival rate of 10^5 cfu/g even after 46 days of inoculation (Table 4).

Change in the Soil Fertility Levels Inoculated with Sand Dune Bacteria: There was increase in pH at 35 DAS with the treatments in the non-sterile soil conditions. The pH of the soil increased from 6.03 at the time of inoculation to 7.50 at the end of the experiment i.e after 35 days of inoculation in *K. rosea* treated soil. After 35 DAS the maximum carbon content increase i.e a 6-fold increase was observed in *Bacillus sp MF-A4* treated soil. There was an increase in available N content on application of SDB and a 3-fold increase was observed in *K. rosea* treated soil. When inorganic P content was analysed all SDB treatments increased P availability with *B.subtilis* showed maximum increase in phosphate content from 30 to 50 mg per kg of soil after 35 DAS (Table 5).

In normal soil conditions, where inoculated cultures were grown at neutral pH, the pH of the soil increased from 6.13 to 7.62 in *K. rosea* treated soil. A marked increase in organic carbon content was observed in all the SDB treatments with a maximum of 4-fold increase in *Bacillus sp MF-A4* treated soil. There was also an 1-2 fold increase in available N content in *K. rosea, Bacillus sp MF-A4* SDB treatment except *B. subtilis* and *M. arborescens* treated soils. The phosphate content of the soil increased greatly in *B. subtilis* (34 to 49 mg / kg of soil) treated soils (Table 6).

DISCUSSION

Sand dune ecosystem is unique and the bacterial community in this ecosystem has not been sufficiently explored for beneficial bacteria. In this study, we isolated 400 bacterial isolates from sand dune ecosystem of Goa. The bacteria were screened for useful traits like production of enzymes, siderophores and solubilization of inorganic phosphates. The effective four isolates were identified as *Bacillus subtilis*, *Microbacterium arborescens*, *Bacillus sp.* MF-A4 and

Kocuria rosea. In the past, bacterial groups such as *Rhodococcus* sp. *Alcaligenes* sp. Unidentified *alpha-proteobacterium, Phyllobacterium myrsinacearum, Bacillus halmapalus DSM* 8723 and *Microbacterium terrae* which were capable of degrading petroleum hydrocarbons have been isolated from sand dune soils of Guadalupe, CA [13]. Park *et al.* (2005) studied the bacterial populations associated with two major sand dune plant species, *Calystegia soldanella* (beach morning glory) and *Elymus mollis* (wild rye), growing along the coastal areas in Tae-An, Chungnam Province and identified majority of the rhizospheric and endophytic bacteria belonging to *Pseudomonas* species and they were useful in plant growth promotion [14]. In an other study by Park *et al.* [15] bacteria belonging to genus *Chryseobacterium* of the family *Flavobacteriaceae* were isolated from two sand-dune plant species inhabiting coastal areas in Tae-an, Korea. However, this may be the first report of *Microbacterium arborescens* and *Kocuria rosea* isolated from sand dune ecosystem. In our studies, 16S rRNA sequences were used for identification of the bacterial isolates as done by other researchers.

The plant growth promoting bacteria are known to produce many growth promoting substances like, siderophores [16, 17], IAA [10, 18] and solubilize inorganic phosphate [19, 20] in soil to enhance plant growth. All the four bacteria used in the study produced appreciable quantity of IAA, produced siderophores and solubilized inorganic phosphates. A good plant growth promoting rhizobacteria possess one or more of the above attributes, which was demonstrated by Ahmad *et al.* [10] and Rai [21]. The bacterial isolates used in this study viz. *B. subtilis*, *M. arborescens*, *Bacillus sp.* MF-A4 and *K. rosea* produce enzymes like protease, amylase, tannase, cellulase which could thus be important industrially. All the isolates produced siderophores and solubilized phosphates which could be important for promoting growth in plants.

Since the bacteria were isolated from coastal ecosystem, we evaluated their growth promoting ability under a similar ecosystem. Eggplant was selected as a model plant as it is popularly grown in our area and is a valuable vegetable species. Seed treatment with *K. rosea* showed increased shoot and root length followed by *Bacillus sp MF-A4,* a similar result was also obtained with pre-germinated seeds treated with SDB on germination paper experiment. Early vigour of the seedlings is important for better establishment, which can be assessed by increased shoot and root length.

Study of SDB on egg plant was carried out both on competitive (non-sterile) and non competitive soil (sterilized soil) conditions with all the microbes. Their results indicated no significant difference in growth parameters between sterilized soil and non sterilized soil in most of the observations up to 35 DAS. Hence it was concluded that the efficacy of SDB to be tested in normal soil i.e competitive conditions. It is assumed that since the pH of the soil is near neutral it was decided to grow the SDB under neutral pH conditions in respective media in order to adapt to the soil conditions when treated. Mean of two season data showed that *K. rosea* and *B. subtilis* increased shoot length and weight of the plants consistently up to 44 DAS. However *Bacillus sp* MF-A4 increased the growth significantly from 37 DAS after sowing. *M. arborescens* was effective in the latter stages (at 44DAS).

The difference in the time of growth promotion may be due to the time taken for initial establishment and colonization of root zones by the bacteria and the rhizosphere competence of respective bacteria. *Bacillus* are spore-forming gram positive rod shaped bacteria which are highly tolerant of adverse ecological conditions. Common physiological traits important to their survival include production of multilayered cell wall structure, formation of stress resistant endospores and secretion of peptide antibiotics, peptide signal molecules and, they probably maintain the population. Several species of Bacillus are known to produce toxins that are inhibitory to the growth and/or activities of fungal and nematode pathogens of plants [22]. Growth promoting effects of rhizobacteria have been reported by several workers in many crops; *Bacillus subtilis* on potato and maize [23] Bacillus spp. on barley [24], on pine and spruce seedling growth [25] *Azospirillum* on wheat seedlings [21, 26], *Rhizobium* on pigeon pea [21] and on non-legumes [27, 28], *Microbacterium* on cotton, wheat and maize. Though *P. fluorescens* is a major member of PGPR and has been reported to enhance crop growth, yield and reduce disease in many crops [29, 30, 26]. Our study shows other bacterial species could also be used as effective PGPR in eggplant.

Population sizes of bacteria decline more or less rapidly following introduction into a natural soil, it has been attributed to the scarcity of available nutrient sources to microbes in soil and the hostility of soil environment to incoming microbes due to a myraid of adverse abiotic and biotic factors Devash *et al.* [33] reported survival of the bacterium in autoclaved soils for 6 months. In Switzerland the inoculated bacterium survived for 120 days approximately.

Effect of inoculants on soil pH and nutrient availability was studied once the crop was removed. In all SDB inoculated treatments pH changed from slightly acidic to near neutral or slightly alkaline. Soil pH affects the solubility of soil minerals, the availability of plant nutrients and the activity of microorganisms. In general, pH values between 6 and 7.5 are optimal for crop growth. It has been shown that soil pH controls the microbial community and that bacteria will decrease at low pH [16, 34]. The carbon content increased 2-4 folds during the crop period in SDB treatments. The plant residues entering the soil are the primary source of soil organic matter and come from litter and root material and increase in carbon in the soil is due to microbial activity in soil [35-38] which breaks down the plant and leaf residues thus increasing carbon in the soil. Nitrogen is especially important, if it is reduced then plant growth is also reduced, as the soil microorganisms will utilize the nutrients in dead organic matter before roots can [39]. There was an increase in the nitrogen content of the treated soils over a period of time which could be due to breakdown of humus and other plant debris, while an increase in the phosphate content of the bacteria treated soils over a period can be attributed to the P-solubilizing ability of the bacterial inoculants which solubilize inorganic phosphates present in the soil to readily utilizable forms of phosphate by producing organic acids such as citric acid,oxalic acid and thus increasing the available phosphates in soil.

Among the four SDB, *B. subtilis* increased C, available N content significantly and increased P availability significantly which could be the reason for increased growth by *B. subtilis*. Also growth enhancement by *Bacillus* may be associated to its ability to produce hormones, especially IAA and Siderophore [40, 41]. *K. rosea, M. arborescens* and *Bacillus sp. MF-A4* isolates increased C, available N and P content which manifested in increased plant growth. The consortium of the SDB did not increase the growth significantly compared to individual application and it was identified that some degree of antagonism exist among them when tested under *in vitro* conditions. Hence consortium application using the above cultures may not be recommended.

REFERENCES

1. Arun, A.B., K.R. Beena, N.S. Raviraja and K.R. Sridhar, 1999. Coastal sand dunes - A neglected ecosystem. Current Science, 77: 19-21.
2. Packham, J.R. and A.J. Willis, 1997. Ecology of Dunes, Salt Marsh and Shingle. 1st Edition. Willis, Chapman & Hall.
3. Davison, J., 1988. Plant Beneficial Bacteria. Biotechnology, 6: 282-286.
4. Bloemberg, G.V. and B.J. Lugtenberg, 2001. Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Current Opinion in Plant Biology, 4: 343-403 402, 50.
5. Krieg, R.N. and G.J. Holt, 1984. Bergey's Manual of Systematic Bacteriology. Vol 1. Williams & Wilkins.
6. Sneath, A.H.P., S.N. Mair and E.M. Sharpe, 1986. Bergey's Manual of Systematic Bacteriology. Vol 2. Williams & Wilkins Bacteriology Symposium, Series No 2, Academic Press, London New York.
7. Cappuccino, G.J. and N. Sherman, 1987. Microbiology, A laboratory manual. 4th Edition. Addison Wesley.
8. Schwyn, B. and J.B. Neilands, 1987. Universal chemical assay for the detection and determination of siderophore. Analytical Biochemistry, 160: 47-56.
9. Nautiyal, S.C., 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiology Letters, 170: 265-270.
10. Ahmad, F., I. Ahmad and M.S. Khan, 2005. Indole acetic acid production by the indigenous isolates of *Azotobacter* and Fluorescent *Pseudomonas* in the presence and absence of tryptophan. Turkish Journal of Biology, 29: 29-34.
11. Gali, S.K., G.S. Dasog, C.M. Poleshi, K.M. Anegundi and P.A. Sarangamath, 2000. Laboratory Manual for SAC 302: Soil Fertility(2+1). Department of Soil and Agricultural Chemistry, University of Agricultural Sciences, Dharwad-5.
12. Olsen, S.R. and L.E. Sommers, 1982. Phosphorus. In: A.L. Pageetal, (eds.) Methods of Soil Analysis. Part 2. Agronomy Mongrams 9. 2nd edition. ASA and SSSA. Madison, WI.
13. McCoy, M.M., 2000. Determination of the Presence of the Catabolic Alkane Monooxygenase Gene From Soil Microorganisms Isolated From Coastal Sand Dunes. A Senior Project. Center for coastal marine sciences. California Polytechnic State University. San Luis Obispo.
14. Park, S.M., R.S. Jung, S.M. Lee, O.K. Kim, O.J. Do, H.K. Lee, B.S. Kim and S.K. Bae, 2005. Isolation and Characterization of Bacteria Associated with Two Sand Dune Plant Species, *Calystegia soldanella* and *Elymus mollis*. Journal of Microbiology, 43: 219-227.

15. Park, S.M., R.S. Jung, H.K. Lee, S.M. Lee, O.J. Do, B.S. Kim and S.K. Bae, 2006. *Chryseobacterium soldanellicola sp.* nov. and *Chryseobacterium taeanense sp. nov.,* isolated from roots of sand-dune plants. International Journal of Systematic and Evolutionary Microbiology, 56: 433-438.
16. Alexander, D.B. and D.A. Zuberer, 1991. Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. Biology and Fertility of Soils, 2: 39-45.
17. Kloepper, J.W., J. Leong, M. Teintze and M.N. Schroth, 1980. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria Nature., 286: 885-886.
18. Tsavkelova, E.A., T.A. Cherdyntseva and A.I. Netrusov, 2005. Auxin production by bacteria associated with orchid roots. Microbiology, 74: 46-53.
19. Nautiyal, C.S., S.B.P. Kumar, H. Lal, R. Mondal and D. Verma, 2000. Stress induced phosphate solubilization in bacteria isolated from alkaline soils. FEMS Microbiology Letters, 182: 291-296.
20. Ponmurugan, P. and C. Gopi, 2006. *In vitro* production of growth regulators and phosphatase activity by phosphate solubilizing bacteria. African Journal of Biotechnology, 5: 348-350.
21. Rai, K.M., 2006. Handbook of Microbial Biofertilizers. Food Products Press, The Haworth Press.
22. McSpadden Gardener, B.B., 2004. Ecology of *Bacillus* and *Paenibacillus spp*. In Agricultural systems. Phytopathology, 94: 1252-1258.
23. Woitke, M., Helmut, Junge, H. Wilfried and S. Chair, 2004. *Bacillus subtilis* as Growth Promotor in Hydroponically Grown Tomatoes under Saline Conditions Proc. VII IS on Prot. Cult. Mild Winter Climates Eds. D.J. Cantliffe, P.J. Stoffella & N. Shaw Acta Hort. 659, ISHS.
24. Canbolat, M.Y., B. Serdar, Ã. Ramazan, Å. Fikrettin and A. Adil, 2006. Effect of plant growth-promoting bacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties and rhizosphere microflora. Biology and Fertility of Soils, 42: 350-357.
25. Shishido, M., H.B. Massicotte and C.P. Chanway, 1996. Effect of Plant Growth Promoting *Bacillus* Strains on Pine and Spruce Seedling Growth and Mycorrhizal Infection. Annals of Botany, 77: 433-442.
26. Tilak, R.B.V.K., N. Ranganayaki, K.K. Pal, R. De, K.A. Saxena, C.S. Nautiyal, S. Mittal, A.K. Tripathi and B.N. Johri, 2005. Diversity of plant growth and soil health supporting bacteria. Current Science, 89: 136-150.
27. Antoun, H., C.J. Beauchamp, N. Goussard, R. Chabot and R. Lalande, 1998. Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: Effect on radishes (*Raphanus sativus* L.). Plant and Soil, 204: 57-67.
28. Matiru, V.N. and F.D. Dakora, 2004. Potential use of rhizobial bacteria as promoters of plant growth for increased yield in landraces of African cereal crops. African Journal of Biotechnology, 3: 1-7.
29. Deshwal, K.V., K.C. Dubey and K. Maheswari, 2006. Long-term effect of *Pseudomonas aeruginosa* GRC1 on yield of subsequent crops of paddy after mustard seed bacterization. Current Science, 91: 423-424.
30. Roesti, D., 2005. Bacterial community associated with the rhizosphere of wheat: interactions with arbuscular mycorrhizal fungi and selection of plant growth promoting rhizobacteria for the increase of wheat growth and soil health in Indian marginal rainfed fields. PhD thesis submitted to the University of Neuchatel, Institute of Botany. Switzerland.
31. Van veen, J.A., L.S. Van Overbeek and J.D. Van Elsas, 1997. Fate and activity of microorganisms introduced into soil. Microbiology and Molecular Biology Reviews, pp: 121-135.
32. Tate, R.L., 1985. Soil microbiology. John Wiley and Sons. New York, pp: 64-72.
33. Devash, Y., Y. Okin and Y. Henis, 1980. Survival of Pseudomonas tomato in the soil and seeds of tomato. Phytopathology, 99: 175-185.
34. Arias, M.E., J.A.G. Gonzalez-Perez, F.J. Gonzalez-Vila and A.S. Ball, 2005. Soil health -a new challenge for microbiologists and chemists. International Microbiology, pp: 13-21.
35. Killham, K., 1994. Soil Ecology. Cambridge University.
36. Paul, E.A. and F.E. Clark, 1989. Soil Microbiology and Biochemistry Academic Press.
37. Wardle, D.A., 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soils. Biology Reviews, 67: 321-358.
38. White, R.E., 1997. Principles and practice of soil science. Blackwell Publishers.
39. Miller, R.W. and R.C. Donahue, 1990. Soils - An Introduction to Soils and Plant Growth. Prentice Hall.
40. Hu, X. and G.L. Boyer, 1996. Siderophore-mediated aluminum uptake by *Bacillus megaterium* ATCC 19213. Applied and Environmental Microbiology, 62: 4044-4048.
41. Ito, T., 1993. Enzymatic determination of itoic acid, a *Bacillus subtilis* siderophore and 2,3-dihydroxybenzoicacid. Applied and Environmental Microbiology, 59: 2343-2345.